PROCESS AND APPARATUS ALLOWING THE DETECTION OF THE FORMATION AND DEVELOPMENT OF BIOFILMS IN A CULTURE MEDIUM

[0001] The present invention relates to the area of the detection of the viscosity of a culture medium.

[0002] The present invention relates more particularly to the area of the study of the development of a biofilm in a homogeneous or non-homogeneous culture medium. This biofilm hinders, as it develops, the movement of particles that can move in a magnetic, electrical or electromagnetic field such as particles that are charged electrically (by the presence of positive and/or negative ions) or magnetically or that are magnetic or magnetizable or covered by a magnetic or magnetizable layer.

[0003] In this connection, in the present text the term "viscosity" is to be understood as referring to the degree of liberty of the magnetizable particle in the biofilm. It will be understood from a reading of the present text that the invention does not have as subject matter a measuring of the viscosity of a medium as would have been understood with the term "viscosity" in its common meaning but rather the demonstration of the development of a microorganism by measuring the degree of liberty of one (or several) magnetizable particles whose movement is hindered or not hindered by a biofilm, which is significant itself of the presence or lacking presence of this microorganism in development.

[0004] Likewise, the expression "culture medium" is to be understood as any medium in which at least one microorganism can be present and developed. It therefore concerns a medium that can be natural or synthetic. Thus, e.g., water is included in this definition. In the remainder of the present text the expression "culture medium" or the terms "medium" or "culture" can be used indifferently by referring to this definition.

[0005] Thus, according to the invention the terms "culture medium", "medium" or "culture" denotes a microorganism and the medium in which it is found or possibly only the medium.

[0006] A microorganism is a living microscopic being such as bacteria, yeast and fungi, algae and protists. A microorganism can be unicellular or pluricellular. The larval stages of pluricellular organisms (metazoas) can also be the origin of biofilms.

[0007] The majority of microorganisms (pathogenic or non-pathogenic) have been studied up to the present in their "planctonic" form, free and isolated in a medium (cultivated in suspension or on a selective medium). In a natural medium outside of the laboratory the bacterial populations are found fixed on the support ("sessile" state) and developed in an organized community called a "biofilm". This bacterial community is generally enclosed in a matrix of exopolysaccharides (EPS) limiting exchanges with the surrounding medium (A. Filloux, I. Vallet. Biofilm: "Mise en place and organisation d'une communauté bactérienne" [French – "Placing and Organization of a Bacterial Community". Medicine/Sciences 2003; 19: 77-83).

[0008] When a biofilm develops there is at first an adhesion of the bacteria on a support, then colonization of this support. When the bacteria multiply they rapidly form a film constituted by strata of cellular bodies secreting a sheath of exopolysaccharides that protects them against aggressions of the surrounding medium (Costerton et al. Bacterial Biofilms. Sciences 1999; 284-6). The kinetics of the formation of a biofilm can be subdivided into 5 stages:

- Conditioning of the surface: The organic or mineral molecules present in the liquid phase will be absorbed on the surface in order to form a "conditioning film".
- Adherence or reversible adhesion: The microorganisms present approach the surfaces by gravimetry, Brownian movements or by chemotaxis if they possess flagellae. During the course of this first fixation stage, causing only purely physical phenomena and

weak physico-chemical interactions to occur, the microorganisms can still be readily detached.

- Adhesion: This slower stage caused interactions with stronger energy to occur as well as the microbial metabolism and the cellular appendages of the microorganism (flagellae, pili, ...). Adhesion is an active and specific phenomenon. The first colonizers will attach themselves in an irreversible manner to the surface in particular by the synthesis of exopolysaccharides. This process is relatively slow and is a function of environmental factors and of the microorganisms present.

- The maturation of the biofilm (development and colonization of the surface): After having adhered to a surface the bacteria multiply and regroup in order to form microcolonies surrounded by polymers. The matrix of polymers (or glycocalyx) will act like a "cement" and reinforce the association of the bacteria among themselves and with the surface in order to finally form a biofilm and attain a state of equilibrium. The biofilm generally develops in a tri-dimensional structure that constitutes a confinement site. This microenvironment will be the seat of numerous physiological and molecular modifications relative to the plantonic growth mode. The biofilm formed in this manner will occupy all the surface that is offered to it if the conditions permit it to do so. The maturation of the biofilm is generally correlated with the production of EPS even if certain species of microorganisms do not synthesize or if only few polymers can likewise adhere and form biofilms on the surfaces.

Detachment: Biofilms are structures in perpetual dynamic equilibrium and develop as a function of the support, of the microorganisms and of the environment this development can be expressed by the detachments of cells or of aggregates.

[0009] This release of cells into the liquid medium can allow as a consequence the contamination of the other surfaces and is in general the cause of numerous recurring diseases in a medical environment (source of resistances).

[0010] The nature of biofilms is very varied - some are very rich in ExoPolySaccharide (EPS) and others are principally constituted by bacterial bodies.

[0011] In human health, biofilms are responsible for infections that are becoming more and more difficult to suppress: in the entire ORL sphere (auditory conduit, nasal membrane, conjunctiva of the eye, ...), on the teeth (appearance of tartar, caries, ...), on the bronchi, the lungs (in patients afflicted with mucoviscidosis, ...), in the urogenital tract (...).

[0012] Furthermore, they are the origin of the majority of nosocomial pathologies (more than 10,000 deaths per year) by forming on catheters or implants (cardiac valves, artificial hips, urinary probes, ...) (J.W. Costerton, P. Stewart and E.P. Greenberg, Bacterial Biofilms "A common cause of persistent infections". Science, vol. 284, pp. 1318-1322).

[0013] Biofilms are also present in refrigeration towers, responsible for infection by legionellas.

[0014] They also affect the agrofood industry on account of their implication in cases of food poisoning (formation during ruptures in the cold chain, development on cutting tools, crunching tools and on work surfaces).

[0015] Likewise, biofilms develop in pipes, causing, in particular, corrosion phenomena.

[0016] Biofilms also develop on the surface of immerged objects, such as, e.g., boat hulls, causing problems of fouling (dirtying of the surface of boat hulls due to the colonization of the hulls by various microorganisms).

[0017] It should be noted that bacteria are not alone in creating biofilms: Fungi, algae and Protozoa also organize into biofilms.

[0018] Biofilms are therefore omnipresent in numerous areas, presenting sanitary risks and causing relatively significant damage.

[0019] However, the development and the behavior of these biofilms remains poorly understood due to the fact of their complexity when being studied, although numerous methods for studying the development of biofilms have been implemented.

[0020] The methods for studying biofilms are still principally based on the colonization of pieces of glass or of plastic immerged in a culture medium contained in flasks under agitation in drying ovens in order to subsequently color them crystal violet or to observe them under a microscope.

[0021] There are other more complex detection methods such as, e.g., detections by Microbalance with quartz crystal (Q-CMD, Quartz Crystal Microbalance with Dissipation Monitoring), detections by MTA (Mass Transport Analysis), by UFDR (Ultrasonic Frequency Domain Reflectometry), by PCR in situ (on functional gene Amo A), by FISH (hybridization in situ under fluorescence), by CLSM (Confocal Laser Scanning Microscopy), by PAS (Photo Acoustic Spectroscopy), ...

[0022] Still other methods use particles/magnetic beads covered with lectin, or antibodies for isolating the bacteria responsible for the development of the biofilm, in order to then allow the characterization of these microorganisms by classic methods of immunoanalysis or by molecular biology (hybridization or PCR).

[0023] However, such methods have proven to be difficult to implement and remain relatively onerous. Furthermore, they do not allow a sufficiently probing teaching to be given

about the behavior of the bacteria and therefore about the formation and development of biofilms. In fact, these methods do not allow the development of a biofilm to be followed, whether it is simply constituted by cellular bodies (Listeria type), by EPS (exopolysaccharide) or by an analogous matrix secreted by colonizing microorganisms (Pseudomonas type).

[0024] The present invention relates to a process and an apparatus that allow the detection of the development of the viscosity of a culture medium, homogenous or non-homogenous, cloudy and/or opaque and to the use of this process and/or this apparatus in particular applications.

[0025] The term "non-homogeneous culture medium" should be understood in the present application in its broadest sense. In particular, a non-homogeneous culture medium can consist of a limpid culture medium in which microorganisms developed in suspension.

[0026] French patent application FR 2555316 is known from the prior art. This patent application concerns a process and an apparatus for determining the viscosity of a fluid medium, which process consists in immersing a conductive bead into the fluid medium, in applying a rotating magnetic field substantially centered on the bead, which rotating field is such that the flow of the fluid in contact with the bead put in rotation remains laminar, and in determining a magnitude connected with the couple exerted on the bead by virtue of the viscosity of the fluid medium. Thus, the bead, plunged in a viscous medium, undergoes a moment of braking proportional to the viscosity and assumes a rotation as a permanent speed whose period is also proportional to the viscosity of the liquid medium to be analyzed. The rotation of the bead can be visualized with the aid of diffraction discs obtained by lighting the bead with the aid of a laser beam along its axis of rotation.

[0027] However, such a process is only adapted for an implementation in a homogeneous viscous medium. A culture medium of bacteria is opalescent, cloudy and opaque. Therefore,

this method does not allow a determination of the formation or lack of formation of biofilms in the culture medium.

[0028] The abstract of Japanese patent application JP61161436 also proposes a method for measuring the viscosity of a non-Newtonian fluid based on the principle of magnetic attraction. The method consists in measuring the viscosity by means of the measurement of the displacement and of the displacement rate of a magnetized bar under the effect of a magnetic field.

[0029] The method proposed in the Japanese abstract allows the determination of the characteristics relative to the viscous fluid such as the viscosity. However, the method in question does not allow in any way a reproduction of the behavior of a microorganism such as a bacteria developing in the viscous fluid.

[0030] The present invention therefore has the problem of proposing a process and an apparatus that allow the modeling of the development of biofilms in a non-homogeneous, cloudy and opaque medium corresponding to the culture medium in which microorganisms develop in order to form such biofilms.

[0031] The present invention also has the problem of allowing the modeling of the process of the colonization of a surface by microorganisms.

[0032] The present invention also has the problem of allowing the demonstration of the differences of viscosity in a in a non-homogenous medium and consequently of allowing the modeling of the culture medium in different zones in accordance with the development of biofilms in each zone.

[0033] The present invention also has the problem of proposing a process and an apparatus for the detection of the development of biofilms that is simple to implement, not very onerous and that can be automated.

[0034] In order to achieve this the present invention is of the above-described type and is remarkable in its broadest meaning.

[0035] Thus, the invention has as subject matter a process allowing the measuring of the viscosity of a culture medium of microorganisms 5 comprising the steps consisting successively in:

a) The immersion of at least one particle that is charged electrically, is magnetic or can be magnetized or covered with at least one magnetic or magnetizable layer in this culture,

b) The subjection of this culture to an electrical, magnetic or electromagnetic field, preferably a magnetic field, in such a manner as to put this particle in motion,

c) The optical detection of the degree of liberty of motion of this particle in this culture, preferably by optical measuring, which process does not use a scanning microscope.

[0036] Step b) consists in subjecting this culture either to an electrical field or a magnetic field or an electromagnetic field, possibly applied by impulsion, or to a progressive augmentation of an electromagnetic field or to more complex variations of an electromagnetic field or to a combination of fields.

[0037] The progressive augmentation of the electromagnetic field is obtained according to a particular configuration of the invention by approaching a magnet along a rectilinear or sinusoidal trajectory or even according to an oscillatory motion that can have or not have a

variable oscillation amplitude and a variable frequency. The more complex variations of the electromagnetic field are obtained by rotation or by combinations of movements of a magnetized bar in the proximity of this culture.

[0038] This electric, magnetic or electromagnetic field is advantageously generated by means for generating a field in motion.

[0039] The culture advantageously flows in a constant stream or in a discontinuous stream at given time intervals through an open reactor. This latter configuration is preferred to the extent that it allows an adequacy with the natural conditions of the development of a biofilm.

[0040] According to the invention, as concerns the particle it can be either an electrically charged particle, a magnetic particle, arranged covered with at least one magnetic layer, a magnetizable particle or a particle covered with a magnetizable layer.

[0041] This magnetic particle can advantageously have a size approximately identical to the size of the microorganisms generating the biofilms.

[0042] It is also advantageously possible to use particles of different sizes and/or, also of advantage, of different colors. The smaller-sized particles are immobilized before the larger-sized particles during the development of a biofilm. It is thus possible to characterize more precisely the development of this biofilm or its degradation.

[0043] Likewise, according to an advantageous configuration of the invention this particle generates a signal detectable by this apparatus for the optical detection of motion. This signal can be detected either in an autonomous manner (advantageously by radioactivity) or by the re-emission of energy transmitted in continuous or discontinuous streams (advantageously luminous transmission of energy by laser beam and re-emission of fluorescence).

[0044] This particle is advantageously of the fluorescent, phosphorescent, radioactive or chemo-luminescent type.

[0045] According to a preferred mode of the invention step c) consists in lighting this particle with a light source and in detecting the motion of this particle in this culture.

[0046] In order to do this, this particle can advantageously be fluorescent.

[0047] According to a preferred configuration this particle 3 is configured in such a manner that it is in a stable position at rest (in the absence of a field) in this reactor 1. This particle can advantageously be a particle, e.g., in the form of a hockey puck, with an asymmetric geometry with a plane face, (...).

[0048] Furthermore, according to a particular implementation of the invention this process consists in performing a measuring of the viscosity of this culture according to the process as previously described at a time t=0 corresponding to the seeding of this culture and at least one measuring at a time t of the viscosity of this culture according to the process as previously described, as well as comparing these measurements at t0 and t.

[0049] The process in accordance with the invention allows the measuring of the viscosity of a culture of homogeneous or non-homogenous microorganisms, preferably non-homogeneous ones.

[0050] According to another aspect the present invention has as subject matter an apparatus that allows the realization of the process of the invention as previously described.

[0051] Thus, the present invention has as subject matter an apparatus that allows the measuring of the viscosity of a culture of homogeneous or non-homogenous microorganisms, comprising:

- At least one culture reactor for receiving this culture in order to perform the detection of the formation and of the development of biofilms,
- At least one particle that is electrically charged or is magnetic or magnetizable or covered with at least one magnetic or magnetizable layer, immersed in the culture,
- Means for generating an electrical, magnetic or electromagnetic field, preferably a magnetic field, which field is applied to this particle in such a manner as to put it in motion,
- An apparatus for the optical detection of the motion of this particle, other than a scanning microscope.

[0052] The term "culture reactor" denotes either an enclosure with at least one closed end of the tube type, well, (...) (closed reactor), or an enclosure with two openings for allowing this culture to flow through this enclosure (open reactor).

[0053] According to a first configuration of the invention this reactor has a closed end in such a manner as to form a flat bottom.

[0054] In order to have a stable position at the bottom of the tube when the particle is at rest, that is to say, when no field is generated, the reactor bottom can have one or several cavities or grooves for receiving this particle or these particles.

[0055] According to a second configuration of the invention this reactor has a closed end in such a manner as to form a hemispherical bottom.

[0056] According to another configuration of the invention the reactor can have two open ends. In this configuration this reactor can be configured in such a manner as to allow this culture to flow in a constant stream or in a discontinuous stream at given time intervals.

[0057] As concerns this particle, it is advantageously either a particle that is electrically charged (by the presence of positive and/or negative ions), or a magnetic particle, or a particle covered with at least one magnetic layer, or a magnetizable particle, or a particle covered with at least one magnetizable layer.

[0058] This magnetic particle advantageously has a size approximately identical to the size of the microorganisms that generate biofilms.

[0059] It is advantageously possible to use particles with different sizes and, also advantageously, of different colors. The smaller-sized particles are immobilized before the larger-sized particles during the development of a biofilm. It is thus possible to characterize more precisely the development of this biofilm or its degradation.

[0060] Likewise, according to an advantageous configuration of the invention this particle generates a signal detectable by this apparatus for the optical detection of motion. This particle is advantageously of the fluorescent, phosphorescent, radioactive or chemo-luminescent type.

[0061] Concerning this apparatus for the optical detection of motion, it comprises a light source transmitting in the direction of this particle, and optical detection means allowing the detection of the motion of this particle in the culture. The term "optical detection means" denotes any usable detection means. According to a preferred embodiment macroscopic optical means are concerned. According to a particular mode of the invention the motion of the particle can be visualized directly with the naked eye.

[0062] Within the scope of this detection the illuminated particle can consist of a fluorescent particle or a particle that is black or at least opaque.

[0063] Particles of different colors, different sizes, different densities, different shapes, geometries, different physico-chemical constitutions, different surface states can be used with

advantage in order to multiply the criteria for the characterization of the development of a biofilm.

[0064] Chemical groupings to be tested can be coupled with advantage to the surface of the particle and the anti-adhesion properties of these chemical groupings (mobile particles) can be tested.

[0065] Molecules allowing the characterization of certain categories of microorganisms can be advantageously coupled to the surface of the particles and the adhesion of these categories of microorganisms (immobilized particles) tested.

[0066] This particle can be directly configured to rest in a stable position at rest in the flat bottom of this reactor. This particle can advantageously be a particle, e.g., in the form of a hockey puck, with an asymmetric geometry with a plane face, (...).

[0067] Furthermore, this apparatus can advantageously comprise measuring means for measuring the viscosity of this culture at given time intervals and comparison means allowing the measurements obtained to be compared.

[0068] It is possible in this manner to test the hindrance to the displacement of this particle due to the presence of colonizing microorganisms or of exopolysaccharides or of matrix secreted by the microorganisms in which this particle is encased at different times.

[0069] The invention will be better understood with the aid of the description, given below purely by way of explanation,, of the different embodiments of the invention with reference made to the attached figures.

[0070] Figure 1 illustrates the principle of the detection of the formation and of the development of a biofilm in a tube with a hemispherical bottom.

[0071] Figure 2 represents the principle of the detection of the formation of a biofilm on the bottom of a tube with a hemispherical bottom (or of tubes other than with a flat bottom) (top view).

[0072] Figure 3 represents the principle of the detection of the formation and of the development of a biofilm in a tube with a flat bottom.

[0073] Figure 4 represents the principle of the detection of the formation and of the development of a biofilm in a tube with open ends.

[0074] Figure 5 represents another illustration of the principle of the detection of the formation and of the development of a biofilm in a reactor 1 of the type of a tube 1 with a flat bottom 2.

[0075] Figure 6 represents a variant of the invention shown in figure 5.

[0076] Figure 7 represents a particular application of the invention as it is described in figure 5.

[0077] Figure 8 represents a particular application of the invention in the area of the surveillance of the contamination of pipes, particularly the surveillance of the contamination of valves.

[0078] The general principle for detecting the formation and the development of a biofilm in a culture containing microorganisms takes place as follows.

[0079] One or more particles or beads that are charged electrically, magnetic, magnetizable or covered with a magnetic or magnetizable layer is/are placed in the culture. The composition or the particles can vary on the condition that it is compatible with a reactivity in an electric, magnetic or electromagnetic field. In order to simplify the following description, these particles will only be described in terms of beads.

[0080] These beads are found incorporated little by little in the matrix secreted by the microorganisms until a complete immobilization.

[0081] In the biological process of the formation of the biofilm the microorganisms are immobilized and surrounded in this matrix. They are then concealed, protected from aggressions from the outside medium, whence the origin of observed resistances to antibiotics (nosocomial pathologies). The beads allow this immobilization to be mimicked.

[0082] In order to mimic this immobilization a field generator is approached to these beads. Thus, in the mediums in which no biofilm has developed the beads react to the approach of this generator and move, in general toward the field generator and possibly following the movement of this generator. On the other hand, if the particles are surrounded in the matrix of the biofilm their movement will be checked and even prevented according to the degree of the formation of the biofilm.

[0083] Therefore, the method of the present invention resides in the exploitation of the behavior of beads that can be put in motion under the effect of electrical, magnetic or electromagnetic fields. If the behavior of these beads is hindered by the presence of the matrix composed in the biofilm it is then possible to detect and to visualize their degree of mobility (mobile, semi-mobile, immobile) and consequently to visualize the development of the biofilm.

[0084] Furthermore, this method allows the differentiation of the beads that can be put in motion under the effect of a field and those whose movements are hindered by the presence of the matrix secreted by the microorganisms.

[0085] The detection of the motion of beads in the biofilm is carried out by optical measuring, either by direct illumination or by indirect illumination. In this latter instance the beads used are advantageously fluorescent.

[0086] The bacterial body will be mimicked more or less precisely and the development of the biofilm characterized with new criteria as a function of the selected format of the beads (geometry, size, density).

[0087] The dynamic development of the matrix constituting the biofilm can be followed as a function of the presentation frequency of the field generator and as a function of the field force. Likewise, once a biofilm has been constituted, its degradation can be followed under the effect of a particular treatment.

[0088] It is then possible to analyze the constitution of this matrix with biochemical tests.

[0089] Likewise, the following of the immobilization of the bead by the matrix constituting the biofilm allows the following, by analogy, of the process of the burying of bacteria in this matrix that they secrete.

[0090] In order to test the development of the biofilm at the bottom of a tube, the detection is conducted with particles that are sufficiently dense to sediment on the bottom of this tube. Inversely, the detection is conducted with particles that are not very dense so that they float at the surface of the culture medium in order to be able to study the development of biofilm on the surface (air/liquid interface).

[0091] Moreover, by using the density of the particles, series of detections can be conducted at solid/liquid, liquid/liquid, liquid/gas interfaces.

[0092] The detections can also use particles with different sizes that can also be, e.g., differentiated by different colors.

[0093] Examples of embodiments of this method will now be described. In these examples the microorganisms described are bacteria. It is understood that the following description is applicable to any other microorganism for which the development of its biofilm is to be studied.

However, the size of the beads is advantageously adapted to the size of the microorganisms studied if one wishes to model the behavior of the microorganisms in the biofilm formed.

[0094] Figures 1 to 8 illustrate the principle of the detection of the formation of a biofilm in different tube geometries that receive a culture containing the bacteria to be studied.

[0095] Figures 1 and 2 illustrate in particular the principle of the detection of the formation and of the development of a biofilm in a reactor 1 of the tube type 1 with a hemispherical bottom 2. Figure 1 is an illustration in section and figure 2 is a top view.

[0096] For example, the experiment can be conducted on a plate presenting 96 tubes (or wells) containing 200 μl. In the present example a bead 3 is placed at the bottom of each tube 1. Of course, the process is not limited necessarily to a single bead. A culture medium 4 is then added into each tube 1, which medium is then seeded with a bacterial strain 5 that can develop into a biofilm 6 under standardized culture conditions (temperature, oxygenation, pH, ...).

[0097] A magnet 7 positioned under tube 1 and more particularly under bead 3 is moved at regular time intervals so as to rise up regularly along the wall of this tube 1.

[0098] When bead 3 does not encounter any obstacle in its motion or is not sufficiently hindered in the matrix secreted by bacteria 5 constituting biofilm 6, bead 3 follows the motion of this magnet 7 (figures 1b and 1c or 2b and 2c). When the magnet is removed the bead is no longer subjected to its field and can return to its initial position. On the other hand, when the formation of biofilm 6 is such that the motion of bead 3 is hindered or even prevented, this bead 3 remains immobile at the bottom of tube 1 (figure 1d or 2d). This state thus expresses a development of the extracellular matrix constituting biofilm 6 in tube 1, such that this matrix surrounds bead 1 in the same manner as it surrounds bacteria 5.

[0099] In this example the magnet is manipulated in such a manner as to move bead 3 along the wall of this tube 1. However, it can be advantageous to manipulate the magnet in the direction of bead 3 or inversely to manipulate the tube toward the magnet in such a manner as to move bead 3 according to another trajectory than the wall of this tube 1.

[0100] An optical apparatus advantageously allows the degree of liberty of this bead to be visualized (not shown). This apparatus comprises a light source emitting in the direction of this bead 3 and comprises detection means allowing the movement of bead 3 in culture 4 to be detected.

[0101] When tube 3 is transparent the light source is located under this tube in such a manner as to emit the light beam directly toward magnetic bead 3. The detection means are then arranged above this tube 3. Thus, the detection of the motion of bead 3 is carried out following the movement of the dark spot corresponding to bead 3.

[0102] When tube 1 is of an opaque material such as, e.g., metal, the light source is arranged above this tube in such a manner as to emit the light beam through culture 4 towards magnetic bead 3. As above, these detection means are arranged above this tube. In this configuration these beads 3 are advantageously constituted by a fluorescent material. Thus, when these beads 3 are illuminated via the light source their movement is detected by these detection means by following the movement of the fluorescent spot corresponding to bead 3.

[0103] Figure 3 illustrates a variant of an embodiment of the invention: the detection of the formation of biofilm 6 in a reactor 1 of the type of a tube 1 with a flat bottom 2.

[0104] Bottom 2 of tube 1 is advantageously provided with two adjacent cavities 8, 9. A bead 3 is placed initially in one of these cavities 8. Magnet 7 is then arranged in contact with the other cavity 9. When bead 3 is not hindered in its movement by biofilm 6 it glides from cavity 8

to adjacent cavity 9 (figure 3b). Magnet 7 is then moved under first cavity 8 (figure 3c). Bead 3 glides toward this first cavity 8 under the attraction of magnet 7 with its motion still not being prevented or at least not sufficiently hindered. And the test is repeated at regular intervals until the observation of the total or partial immobilization of the beads 3 as illustrated in figure 3d: when magnet 7 is moved under the second cavity 9, bead 3 stuck in biofilm 6 can no longer pass in response to the attraction of magnet 7 into second cavity 9 due to the fact of the hindering of its motion in this biofilm 6.

[0105] In a variant the tube bottom does not have cavities for receiving the magnetic bead or beads. To this end this magnetic bead is configured so as to be able to maintain itself in a stable position at the bottom of this tube 1.

[0106] Figure 4 illustrates another embodiment of the invention using a reactor 1 of the tube type 1 with two open ends 10, 11. Tubes 1 is then configured to permit a continuous stream of culture medium 5.

[0107] As in the example of the tube with a flat bottom, inner surface 12 of wall 13 of this tube 1 advantageously has cavities 8, 9 for receiving this bead or these beads 3. According to the same principle as the one previously described, magnet 7 is presented in such a manner as to put beads 3 in motion in such a manner that they pass from one cavity to the other.

[0108] In the instance in which no cavity is formed in inner surface 12 of wall 13 of this tube 1 the principle will be similar to the one described for the tube with a hemispherical bottom: magnet 7 is presented and moved in such a manner as to bring these beads up on inner face 12 of wall 13 of this tube 1.

[0109] According to a particular configuration of the invention the beads encased in biofilm 6 can be subsequently recovered by a magnet being immersed into the culture. In this manner a

fragment of the biofilm is taken for tests of physical characterization (viscosity of the matrix, etc.), chemical and biochemical characterization (constituent elements of the matrix, etc.), and biological characterization (microorganisms constituting the matrix in a state of latency, inactivity, dead bodies, etc.).

[0110] Figure 5 is another illustration of the principle of the detection of the formation and development of the biofilm in a reactor 1 of the tube type 1 with a flat bottom 2. This illustration is a plane view from the top of the tube.

[0111] Beads 3 are placed at the bottom of each tube 1. A culture medium 4 is then added into each of the tubes (figure 5a), which medium is then seeded with a bacteria strain 5 that can develop into biofilm 6 (figures 5b to 5e) under standardized culture conditions (temperature, oxygenation, pH, ...).

[0112] A magnet 7 is positioned at regular time intervals under tube 1 (figures 5b and 5e). When beads 3 do not encounter an obstacle in their motion or are not sufficiently hindered in the matrix secreted by bacteria 5 and constituting biofilm 6, they are attracted in the direction of magnet 7 (figure 5b). Beads 3 attracted around magnet 7 free a zone "without beads" or "clear zone" that is simple to detect, particularly visually. When the formation of biofilm 6 is such that the motion of beads 3 is hindered or even prevented, these beads 3 remain immobile at the bottom of tube 1 (figures 5d and 5e). This state then expresses a development of the extracellular matrix constituting biofilm 6 in tube 1 such that this matrix surrounds magnetic bead 1 in the same manner as it surrounds bacteria 5.

[0113] Figure 6 illustrates a variant of the invention shown in figure 5.

[0114] Petri dishes 1 containing a liquid culture medium 4 are seeded with bacteria 5, and magnetic beads 3 and 3' of different sizes are placed in each dish 1 (figure 7). The culture

conditions are standardized (temperature, oxygenation, pH, ...) in order to allow the development of bacteria and therefore the development of biofilm 6.

[0115] Magnet 7 is positioned under Petri dish 1 at regular time intervals. When bead 3 does not encounter an obstacle in its motion or is not sufficiently hindered in the matrix secreted by bacteria 5 and constituting biofilm 6, magnetic beads 3 are attracted in the direction of magnet 7. A clear zone 14 then develops between the outer limit of the influence zone of the magnetic field lines 9 that attract the beads and the aggregate of the beads 15. When the formation of biofilm 6 is such that the motion of beads 3 is hindered or even prevented, these beads 3 remain immobile in dish 1. However, due to the fact of the difference in size of the beads their movement is a function of their size and of the density of the biofilm. As the biofilm develops the small beads will have their movement inhibited by the biofilm first, then, with a supplementary development of the biofilm the large beads will be stopped in their turn.

[0116] Figure 7 illustrates a particular application of the invention as it is described in figure 5 or in figure 6 with the beads placed on a surface covered with a product containing an anti-microbial agent such as, e.g., an anti-fouling agent. This surface can be of any material, in particular of metal. When a magnet is approached to the surface the beads are attracted by the force lines of the magnet, that then constitute a bead density zone larger than on the rest of the surface. This application is advantageous when it is desired to measure the effectiveness of an anti-fouling product applied on a metallic surface.

[0117] In this particular embodiment it can be more interesting to vary the intensity of the magnetic field, e.g., by rotating a magnetized bar under the surface to be tested.

[0118] Figure 8 illustrates a particular application of the invention in the area of the surveillance of the contamination of pipes, particularly in the surveillance of the contamination of valves.

[0119] In order to model the development of biofilm on a support subjected to a liquid stream (pipes 1), it is possible to use an apparatus with an annulus 16 held in a bulge of a tube 17. A magnetizable particle 4 is enclosed at a point of annulus 2. This annulus can be rotated under the action of a magnetic field (figure 8b or 8c).

[0120] If a biofilm develops in the apparatus the motion of the annulus is hindered.

[0121] This apparatus models a valve, the site in pipes where biofilms develop most readily.

[0122] The invention is described above by way of example. It is understood that an expert in the art is capable of realizing different variants of the invention without departing from the scope of the patent.